SEEDLING DISEASE OF SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH]: EPIDEMIOLOGY, ETIOLOGY AND RESISTANCE

A Thesis

by

GREGORY ALLAN FORBES

Submitted to the Graduate College of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 1984

Major Subject: Plant Pathology

SEEDLING DISEASE OF

SORGHUM [SORGHUM BICOLOR (L.) MOENCH]:

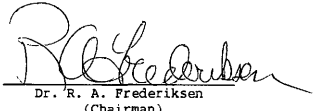
EPIDEMIOLOGY, ETIOLOGY AND RESISTANCE

A Thesis

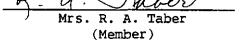
by

GREGORY ALLAN FORBES

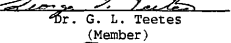
Approved as to style and content by:



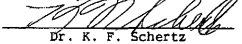
Dr. R. A. Frederiksen
(Chairman)



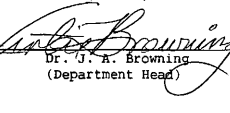
Mrs. R. A. Taber
(Member)



Dr. G. L. Teetes
(Member)



Dr. K. F. Schertz
(Member)



Dr. J. A. Browning
(Department Head)

August 1984

ABSTRACT

Seedling Disease of

Sorghum [Sorghum bicolor (L.) Moench]:

Epidemiology, Etiology and Resistance (August 1984)

Gregory Allan Forbes, B.A., Murray State University, Murray Ky.

Chairman of Advisory Committee: Dr. Dr. R. A. Frederiksen

Sorghum seedlings were evaluated in the greenhouse under various temperature (10 - 25C) and moisture (-1 - 0 bars matric potential) regimes in both non-pasteurized field soil (NFS) and pasteurized field soil (PFS). Three dependent variables were measured: leaf length, leaf weight and final seedling emergence.

In the NFS, the moisture factor produced the most important main effect on all variables measured, though certain temperature by moisture interactions produced small but significant effects on some or all variables. Generally, neither moisture nor temperature had deleterious lasting effects on seedling growth, in the PFS. The absence of temperature and moisture effects in the PFS suggested that pathogenic interactions are involved in the sorghum stand establishment problem.

Based on the frequencies of species isolated from diseased tissue and reports in the literature, Fusarium moniliforme, Rhizoctonia solani and Pythium arrhenomanes were chosen for pathogenicity tests. NFS and PSF were infested at 3 inoculum densities (1/10, 1/100 and

1/1000; V/V) with one isolate of each fungus. In PFS, P. arrhenomanes reduced leaf length, final emergence and leaf weight of sorghum seedlings at the highest inoculum density (ID) and only leaf weight and leaf length at the lower ID. R. solani reduced leaf weight and leaf length only at the highest ID. F. moniliforme had no apparent effect on any variable at the inoculum levels tested. In NFS, none of the isolates caused discernable effects on sorghum seedlings at any ID tested.

Nine agronomically important genotypes of sorghum were evaluated in the field for postemergence damping off. These genotypes, plus 3 more, were evaluated in the greenhouse using three techniques. QL3, BTx378 and SC630-11E were resistant in all or 3 of the tests, suggesting the existence of heritable traits of resistance to seedling diseases.

DEDICATION

I would especially like to thank Dr. R. A. Frederiksen, my committee chairman and project leader, for making this research opportunity possible, and for providing suggestions and resources whenever needed. The freedom that he gave me to direct much of my own research was intimidating at first, but certainly added another dimension of learning to my education.

I also wish to thank my committee members, Mrs. R. A. Taber, Dr. G. L. Teetes, and Dr. K. F. Schertz, for their constructive and encouraging comments on my proposal and thesis manuscripts. The helpful hints which Mrs. Taber gave me regarding the isolation and identification of soil-borne fungi were also extremely valuable. In addition, I would like to thank Dr. G. N. Odvody for sharing with me his ideas and observations on seedling disease of sorghum.

TABLE OF CONTENTS

Table of Contents (Continued)

Table of Contents (Continued)

LIST OF TABLES

List of Tables (Continued)

CHAPTER I

INTRODUCTION

Problem Status

Sorghum [Sorghum bicolor (L.) Moench], seedlings display relatively high mortality in the first 1 1/2 months after planting in the field. The difference between field stand percentages and higher laboratory germination percentages is greater than for most other crops (42). When conditions are less than optimal, stand establishment becomes an even greater problem. Since the threat of midge damage and moisture stress decreases with early planting of sorghum, commercial seed companies and public institutions are currently trying to develop new cultivars with the ability to establish a good stand in cool, wet soils.

While the cause of reductions in emergence percentages of sorghum seedlings has been studied for half a century, no holistic approach has yet been employed in an attempt to determine the major effects and interactions of both abiotic and biotic factors. Investigations have focused on temperature, moisture, soil-borne or seed-borne organisms or some combination of these. In all cases, however, one or several factors of potential importance were either not properly controlled or were ignored. Consequently, there is no clear documentation in the literature of the effects of soil or seed-borne microorganisms on

---

The journal used as a style pattern was Phytopathology.

sorghum seedling establishment under suboptimal conditions. Neither has it been conclusively established that temperature (in the 15-25 C range) has a direct effect on final seedling emergence.

Therefore, prior to screening sorghum seedlings for stand establishment potential under adverse conditons, the following questions had to be addressed. First, how do certain soil moisture and temperature levels affect seedling stand establishment (final emergence percentage and seedling survival)? Second, are these temperature and moisture effects direct, or do they influence the interaction between the seedling and the soil microflora? This research was designed, in one or more ways, to address these questions.

Literature Review

Researchers have generally followed one of two approaches in the study of factors which may influence stand establishment. Some have explored the abiotic physiological effects of variation in moisture and temperature on seedling growth, ignoring or excluding the effects of the soil and seed-borne microflora. Other researchers have investigated the interaction between certain soil or seed-borne fungi and the plant, under conditions which are not representative of the stand establishment problem as it is in nature. They have ignored or excluded potential moisture and/or temperature effects. Though a few researchers have attempted to integrate the abiotic and biotic approaches, one or more important factors of potential importance have always been ignored.

The Abiotic Approach

In one of the earliest reports of a stand establishment problem, Harris and Goss (15) investigated a "reddening of the primary roots and particularly of the mesocotyl. In a short time after the red condition appeared, the tissue began to shrivel and darken." Diseased plants were sometimes killed, but other plants often recovered in the field. The environmental conditions associated with the disease were not described. Seeds were surface sterilized and germinated on agar slants. Ninty percent of the seedlings which had no signs of fungal growth, developed red mesocotyls and roots. Subsequent pathogenicity tests in sterile sand, demonstrated a relationship between root reddening on the agar slants and susceptibility to Fusarium sp. for some genotypes. The authors concluded that the reddening was physiological and that it rendered the seedlings more susceptible to attack by the fungus.

Anderson (2) later determined that the reddening of sorghum seedlings grown in agar slants was temperature dependent. At 15 - 20 C seedlings did not redden even after 20 days, while at higher temperatures the symptoms began to develop within several days.

Though not suggested by either Anderson or Harris and Goss, one plausible reason for the reddening is the accumulation of toxic levels of root exudates in the agar. Higher temperatures would increase growth and, therefore, the amount of exudates in the tubes.

Martin et al (25) found that the rate of emergence and the percent emergence of seedlings grown in sterile soil were lower at 15 C than at 25 C. Yet, they did not investigate the possible effects of

soil moisture or of the introduction of seed-borne organisms into the sterile soil.

Evans et al (12) used a factorial experiment to study the effect of moisture and temperature on what was termed "sorghum seed germination". In the experiment, however, rate and percent emergence (after 10 days) were measured instead of germination. The soil was adjusted to 3 moisture levels: 10, 15 and 24% (moisture/dry soil wt). The levels were chosen to study the limiting effects of moisture on seedling germination, emergence and growth. The wettest level, which was near field capacity for that particular soil, was below the levels that would be found in the field after a rain.

The microfloral constitution of the "oven dryed" (sic) soil that was used in this experiment is not known. Most soil-borne pathogens die at relatively low temperatures (5), and the drying procedure may have pasteurized or sterilized the soil.

The temperature and moisture levels utilized by Evans et al (12) were found to significantly affect the rate of seedling emergence and the final percent emergence. Yet, little can be inferred by the temperature effect on percent emergence. The authors commented that the 10 day duration of the experiment was not sufficient for emergence to be completed at the lowest (18 C) temperature. Thus, differences in the number of seedlings emerged between the lowest and highest temperatures may have little bearing on the final emergence percentages.

The philosophy which has given impetus to the abiotic approach was represented by Stoffer and Van Ripper (42) who wrote, "an

understanding of the influence of variable environmental factors on the physiology (my emphasis) of the sorghum plant.....could lead to the elucidation of the soil temperature and soil moisture levels necessary for optimum sorghum stand establishment, growth and development."

Stoffer and Van Ripper (42) demonstrated that field-planted seedlings had a higher percent emergence when minimum soil temperatures reached 18 C. Soil moisuture was monitored in the field, but no mention was made of the effects of soil moisture on emergence.

In controlled environment studies, the effects of various moisture and temperature levels on final emergence and other variables were evaluated. Data for final emergence were not presented, leaving the reader with the uncertain assumption that the effects of temperature and moisture were not statistically significant. The moisture levels employed were in the dry range and were chosen to test the effects of moisture availability on germination and emergence. The wettest treatment was at 100% of "available moisture" (15.5% moisture/dry soil wt.).

Refering to the field data, the authors suggested that "soil temperature would be a better criterion for determining the time for planting grain sorghum during the spring than a specific calendar date, if soil moisture and other environmental conditions were favorable." In these experiments, the possible effects of the soil microflora on stand establishment were not investigated.

Blum (6) noted that sorghum X sudangrass hybrids had higher emergence percentages at 15 C than did the parents, but not at 28 C.

The author suggested that the reduction in percent emergence below 28 C of the sorghum and sudangrass varieties was "not associated with any apparent attack by parasitic organisms". He did not document, however, how he confirmed the absence of parasitism in the sterile sand used in the study. Sorghum seeds invariably contain a fungal microflora. Many of these fungi, though not considered parasites, cause considerable damage to seedlings in sterile sand where they are free from competition with other organisms. Differences in seed microflora among varieties or hybrids might cause differences in emergence potentials, which are accentuated by adverse conditions.

Horrocks (18) devised a model for sorghum emergence using variation in planting depth and soil temperature as independent variables. Soil moisture and soil microflora were not considered. The model predicted emergence percentages which were too high for the low temperatures and too low for the high temperatures. More recently Monk (29) designed a model for seedling emergence which incorporated soil moisture, soil temperature and planting depth. Moisture levels were basically in the dry range and demonstrated the low limits at which moisture availability affected emergence. High moisture levels, such as often occur with early spring plantings, were not considered. The effects of low moisture levels on seedling emergence were also studied by Stoffer and Van Ripper (42). Several researchers have demonstrated or observed deleterious effects of moisture or temperature on seedling emergence percentage, but have not attempted to explain the effects in terms of its direct or indirect nature (43,16,32,22).

The Biotic Approach

The second general approach taken by researchers has been to evaluate the interaction between seedlings and soil and seed-borne organisms. In the 1930's, Pythium arrhenomanes, then the suspected causal agent of Periconia crown rot (11), was tested for pathogicity on sorghum seedlings. The organism was shown to cause reductions in percent emergence in sterile soil. At the time, no formal connection was made between the pathogenicity of the fungus in sterile soil and its potential role in stand reduction in the field.

Since then, isolates of soil and seed-borne fungi have repeatedly been tested for pathogenicity on sorghum seedlings. While exhaustive literature reviews are given by Castor (7), Williams and Rao (47) and Pratt and Janke (35), a few articles are mentioned here to demonstrate that many types of fungi have been shown to be pathogenic on sorghum seedlings under conditions much different from those found in the field.

Porter reported a species of Rhizopus pathogenic on sorghum seedlings in sterile sand (34). Freeman et al (13) showed that Pythium aphanidermatum reduced seedling survival percentages in fumigated soil. Helminthosporium and Fusarium sp. (21) caused measurable damage to sorghum seedlings when spore-infested seeds were germinated in petri dishes. In sterile soil, Rhizopus sp., Alternaria sp. and Aspergillus sp. (31), and Pythium aphanidermatum and P. myriotylum (27) caused pre or postemergence seedling death.

Pratt and Janke (35) tested three species of Pythium in both field and sterile soil. P. graminicola was generally more pathogenic

than P. myriotylum or P. periplocum. Environmental influences were not considered.

Integrating the Biotic and Abiotic Approaches

Several researchers have attempted to determine whether soil temperature directly or indirectly affects seedling emergence and, therfore, stand establishment. The effects of different temperature levels on sorghum seedling (measured by percent emergence and post emergence damping off) were studied in both steamed (sterile) and field soil by Leukel and Martin (23). They demonstrated that the adverse effect of suboptimum temperatures on seedling emergence and survival was greater in field soil than in steamed soil. The slight reduction in percent emergence at the lowest temperature (15 C) compared to the highest temperature (26 C) in the steamed soil was attributed to the introduction of seed-borne fungi into the growth medium. Based on numerous observations and pathogenicity tests, they concluded that soil-borne Pythium spp. were the primary pathogens responsible for reductions in percent emergence and seedling survival. Unfortunately, no attempt was made to study the effects of variation in moisture levels.

The results of Leukel and Martin were reinforced by experiments conducted in Israel by Pinthus and Rosenblum (33). They demonstrated that temperature had only a slight deleterious effect on seed germination in rolled towels, and that this effect was magnified when soil was added to the seed. Both the slight temperature effect in the absence of soil, and the major temperature effect in the presence of

soil were reduced by fungicidal seed treatment. Seed and soil-borne organisms were implicated, though the latter were believed to be more important under the conditions of the test.

Further indirect evidence for the implication of soil-borne organisms in the deleterious effects of low temperature on sorghum seedling emergence was provided by Josifovich (20). He found that there was a greater temperature effect when seedlings were planted in field soil than when germinated on filter paper in petri dishes. Different moisture levels were not tested.

Mustain (30) attempted to determine whether the negative effects of low and high temperature on emergence percentages of sorghum seedlings were direct or indirect, by growing both inoculated and uninoculated seed in sterile sand. He hypothesized that this question could only be answered if one could produce "fungus-free" seed. Though he was unable to do so, he concluded from several emergence tests, using relatively clean seed, that the "direct negative effect of cool temperature (on percent emergence) is weak if it exists at all,...."

The role of micro-organisms in seedling emergence and stand establishment problems has been indirectly implied by several field fungicide studies. The difference in percent emergence between treated and non-treated seed has been shown to be most significant with early plantings (14,16,24,8). These data do not preclude the direct deleterious effects of low temperature, but do suggest that at least some of the effect is due to soil or seed-borne fungi.

Etiology

As illustrated above, only the effects of low soil moisture on percent emergence have been addressed thus far. Field observation would suggest, however, that high levels of soil moisture are also important in stand establishment. When stand establishment problems occur in the field, soils are very wet. Severe seedling stand reductions in South Texas in 1983 resulted when the area experienced rains and cold temperatures soon after planting. During this period, soils were probably saturated or flooded for hours or days at a time.

Part of the importance of soil moisture may be due to the type of organisms which cause seed rot or seedling disease. Leukel and Martin (23) concluded that soil-borne species of Pythium were the major causes of seedling disease. They isolated more species with spherical sporangia than any other type. Species with lobulate sporangia, such as P. graminicola and P. arrhenomanes have been found in association with diseased sorghum seedlings (40,26) and shown to be pathogenic in greenhouse experiments (26,35). Some spherosporangial species germinate with a germ tube and may require less moisture for infection than species producing zoospores. Studies indicate that the optimal matric potential for zoospore release for several species of Pythium and Phytophthora is near 0 bars (9). Screening techniques which utilize dryer soils would be biased in favor of fungi whose dispersal mechanisms have less exacting requirements with respect to soil moisture matric potential.

The role of Fusarium spp. has been clouded somewhat by their association with the seed before it is put in the ground. F.

moniliforme, potentially affects seedling emergence and vigor in two different ways. It may affect seedling vigor because of damage done prior to sowing, having killed or weakened the embryo or depleated resources in the endosperm. As a superficially seed-borne organism, the fungus may attack the seedling only under certain conditions, its pre-sowing association with the grain being important in that it puts the fungus in close proximity to the germinating seed. Since little is known about the ability of F. moniliforme to colonize healthy seedling tissue with no prior damage to the kernel, it is difficult to say whether the fungus reduces seedling stands solely because of its effect on seed quality or because it also acts as a true seedling disease pathogen. While most pathogenicity studies with F. moniliforme have been conducted in sterile soil or potting media (15,23,30), Junejo and Malet (21) did notice reductions in emergence and seedling dry weight after 1 1/2 months when seeds were superficially inoculated with a spore suspension and planted in the field.

CHAPTER II

MATERIALS AND METHODS

General Procedures

Response Variables

Leaf length, dry leaf weight and final emergence were the dependent variables measured in several experiments. Leaf length refers to the distance in cm from the first node to the farthest tip of the foliar part of the seedling. Dry leaf weight (referred to in the text simply as "leaf weight") represents the dry weight in g of the foliar part of the seedling, excluding the mesocotyl. To make this measurement, the tissue was dried in a forced-air oven (80 - 90 C) for approximately 24 h. Variation in drying time occurred only between experiments and not within experiments. Final emergence refers to the number of plants emerged after a specified amount of time and should not be confused with percent emergence.

Soils and Soil Preparation

Experiments were conducted using either a Houston Black Clay soil from Temple, Texas, or a red clay loam (Norwood series) from the Texas A&M Experiment Station on the Plantation Farm at College Station, Texas. Soil moisture release curves were run for both soils (Table 1).

Soils were tested in the matric potential range between -1 and -.1 bars on a pressure plate extracter, and between -.1 and 0 bars on

Table 1. Matric potential and corresponding values of percent moisture for two soils used in greenhouse and incubators

Matric potential (-bars)	Percent moisture[1]	
	HBC soil[2]	Norwood soil[3]
.025	47	41
.045	42	35
.075	41	-
.10	38	29
.50	33	25
1.0	29	23

[1] Percent moisture relative to soil dry weight.
[2] Houston Black Clay soil.
[3] Brazos Bottoms soil (Norwood series).

matric potential tension table. Both procedures are explained in detail by Richards (36).

Pasteurized field soil (PFS), used in several experiments, was prepared in the following manner. Non pasteurized field soil (NFS) was put in burlap sacks and steamed without pressure in a wooden box for 50 - 60 min. Immediately upon removal from the steambox, the soil temperature was approximately 95 C. Repeated isolation attempts from aseptically removed soil samples always yielded copious bacterial growth, but no fungi were encountered. It was assumed that only the spore-forming bacteria were able to surivive the steaming treatment.

Isolations

One general procedure was followed for all attempts to isolate from plant tissue. Plant pieces were cut to approximately 1 cm in length and washed repeatedly in tap water to remove surface debris. The tissue was then surface sterilized in a 5-10% solution of commercial bleach (containing .05% sodium hypochlorite), and aseptically patted dry on sterile filter paper. The pieces were then submerged into the agar. This technique favors fungi over bacteria by reducing free moisture on the surface of the tissue.

Epidemiology

Complete Moisture and Temperature Effect

Experimental Design. Moisture and temperature were main factors in a factorial experiment. Experimental units, each consisting of one tray (described below) were replicated 4 times and randomly assigned positions in the temperature tanks (described below) in a completely randomized design.

Materials and Procedures. Thirty seeds of sorghum hybrid Funks G611 were sown 4.5 cm deep in Brazos Bottoms clay loam soil in metal trays (6 cm wide x 11 cm deep x 32 cm long), which were suspended in three water tanks. The tanks, which were located in the greenhouse, were regulated to 15, 20 and 25 C. An additional temperature level was created by diurnally rotating between the 10 and 25 C tanks (approximately a 12:12 hr rotation).

The following three moisture levels were used:

(1) Dry = soil brought to approximately -0.5 bar matric potential at the time of planting, and not irrigated for 20 days.
(2) Wet = soil brought to saturation at the time of planting and not irrigated for 20 days.
(3) Saturated = soil brought to saturation every 48 hr for a 20 day period.

A 3 cm layer of "pea" gravel (mean dia. approximately 0.5 cm) was placed in the bottom of each tray before adding the soil. A piece of plastic pipe 2.5 cm in dia. was pushed to the bottom of the gravel such that the top of the pipe extended about 2 cm above the top of the tray. When water was added to the soil in the tray, the excess collected in the gravel below the soil and could be evacuated through the plastic pipe with a 10 ml pipet connected to a vacuum pump.

The dry and wet treatments, which were not watered while in the tanks, were covered with loosely fitting plastic to retard evaporation.

All the trays were taken out of the tanks after 20 days and randomly placed on a greenhouse (22-28 C) bench. Moisture was maintained uniformly for 11 days, after which time leaf length, leaf weight, and final emergence were measured.

Comparing the Biotic and Abiotic Components

Experimental Design. Moisture and soil type were the main factors in a factorial experiment. Experimental units, each consisting of one cup with soil and seedlings, were replicated 4 times and randomly located in the temperature chamber in a completely randomized design.

Materials and Procedures. Seeds of sorghum hybrid Funks G611 were pregerminated at 27 C for 24 h before planting. Moisture levels were obtained by adjusting watering regimes in the following manner. At the time of planting, soil was brought to approximately - 1 bar matric potential. One fourth of the experimental units were left at this moisture level until taken out of the temperature chamber and were called the "moist" treatment. Another group (1/4 of the total) of units was brought to saturation at the time of planting and not watered again until removal from the temperature chamber. This treatment was referred to as "wet". The "saturated" treatment consisted of maintaining the units near saturation throughout the period they were in the temperature chamber, by adding approximately 45 ml of distilled water daily. This amount resulted in consistant runoff, which was interpreted as an indication that the soil had reached saturation. On several occasions, units with no seeds were tested gravimetrically for moisture level and were found to be at, or very near saturation.

The "flooded" treatment was similar to the "saturated" treatment except that 25 h of flooding was initiated immediately after planting. The containers were flooded with distilled water so that the soil was covered by approximately .5 cm. The cups remained in an incubator (10 - 20 C) for 6 days and were then moved to a greenhouse bench and maintained at approxiamtely equal moisture levels. After 13 days in the greenhouse, the seedlings were measured for leaf length, leaf weight and final emergence.

Measuring the Biotic Component

Experimental Design. Moisture and temperature were the main factors in a factorial experiment with paired differences. Each experimental unit consisted of one NFS and one PFS subunit. Experimental units were placed in the temperature chambers in a completely randomized design. Leaf weight, leaf length and final emergence were measured as the difference between the PFS and NFS subunits, for each experimental unit.

Materials and Procedures. Sorghum seedlings were subjected to various pre-emergence temperature and moisture levels in both field and pasteurized soil. Temperature levels were 10, 15, 20, 25 C and one 10 - 20 C diurnal cycle (12:12 h). These were controlled by 4 incubators and 1 inoculation chamber. Temperatures were monitored daily, and were consistantly found to be within one degree of the desired temperature.

Moisture levels were obtained by adjusting watering regimes in the manner described for the preceeding experiment with one modification. Flooding was initiated 48 h after planting and lasted for 24 h.

Ten seeds (without pregermination) of the sorghum hybrid Funks G611 were sown at a depth of 4.5 cm in either pasteurized or non pasteurized Houston Black Clay soil in 190 ml stryrofoam cups. After moisture adjustments were made in the manner described above, all experimental units (each consisting of two cups, one with field soil and one with steamed soil) were randomly placed in their prospective temperature chambers. When seedlings began to emerge in 4 cups in a

particular chamber, all the cups in that chamber were moved to a bench in the laboratory (23 - 25 C) where they were illuminated by florescent and grow (Sylvania Gro Lux) lights. The cups in the 10 C chamber were removed arbitrarily after 18 days, because no seedlings had yet emerged. Moisture was maintained at approximately the same level in all units while they were on the bench. This required differential watering regimes, due to the different rates of transpiration among treatments. The experimental units for each temperature level (all the cups for one temperature chamber) were left on the bench for 19 days, after which time leaf length, leaf weight and final emergence were measured.

Durations of the pre-emergence temperature treatments were as follows:

10 C = 18 days

15 C = 10 days

10-20 C = 7 days

20 C = 5 days

25 C = 4 days

Etiology

Isolations from Seedlings Grown in the Greenhouse

Though several isolation experiments were conducted in the greenhouse, two representative examples are presented.

Experiment one. Seedlings were grown in a temperature tank at 15, 20 and 25 C in Houston Black Clay soil. Moisture was maintained at uniform high levels in all temperature tanks. After 20 days,

seedlings were removed from the trays and root systems were washed free of soil with tap water. One cm pieces of primary and adventitious root tissues were surface sterilized and plated on 4 media in the manner described in the General Procedures (p.). The media used were: 1) RBS (Rose Bengal agar with 200 ppm streptomycin); 2) CPV (Cornmeal agar with 100 ppm pimarcin and 10 ppm vancomycin); 3) NS (Nash Snyder agar), and; 4) Water agar. CPV is selective for phycomycetious fungi (45). NS is selective for Fusarium spp. (7). RBS reduces the rate of fungal growth (45), and water agar has no particular reported selectivity.

Five 1 cm pieces of tissue were put on each plate. Seven plates of each medium were used for each of the three temperatures. Isolates were identified on the original plates or transferred to water on which a piece of sterile agar had been placed (41). This agar proved to be the most useful, both for cultural characteristics (inducing the production of specialized structures) and in viewing the fungi.

Experiment two. Seedlings were grown in the temperature tank in the manner described above and rotated daily between the 15 and 25 C tanks. Seedlings were removed and cleaned after adventitious roots were 5 to 15 cm long. Tissue with lesions or reddening from primary roots was plated on the four media described above. Tissue with lesions or reddening from the adventitious roots was plated on water agar only. Fungi were identified on the original plates, or transferred to water agar, which had been ammended with a piece of sterile oatmeal.

Isolations from Seedlings Grown in the Field

On several occasions in the spring of 1983, diseased seedlings were collected in south Texas. In each case, the materials were maintained on ice until isolations were made. Isolations on water agar were done according to the method described in the General Procedures (pg. 12).

Histology

Several very simple histological techniques were employed to help identify which organisms were attacking the subterranean parts of the seedlings. Techniques utilized included: squashes of diseased tissue, 50 - 75 micron sections cut with a kryotome, or 8 - 15 micron sections cut on a rotary microtome after embedding in paraffin.

In Vitro Pathogenicity Test

Experimental Design. Fungal species was the single factor in a completely randomized design. Five different species and one control were replicated 5 times for a total of 20 experimental units.

Materials and Procedures. Three pregerminated seedlings of sorghum line BTx623 (apparently free of fungal growth) were placed in each of 24 blotters in a blotter inoculation apparatus. The apparatus consists of a series of paper blotters (3.5 cm wide x 6 cm long) suspended in a container. The blotters are cut from paper towels (Plyfold, No. 226 Garland Towels. Ft. Howard Paper Co., Green Bay, Wi.) The blotters extend down into a reservoir of water at the bottom of the container and remain wet through capillary water movement.

Seedlings grow between two blotters, which are stapled together. Inoculum can readily be placed on or near any part of the growing seedling. More details of the procedure are given by Singleton and Ziv (38).

After incubation for 48 h in the blotter apparatus, the seedlings were inoculated with .5 cm plugs of 7-day-old cultures of Fusarium moniliforme, Fusarium sp., Pythium arrhenomanes and Pythium sp. (lobulate). One plug was placed approximately 2 cm from the root tip of each inoculated seedling. After three days, root systems were evaluated for symptoms of disease (reddening, watersoaking or necrosis) and leaf length was measured from the mesocotyl to the most distal foliar point.

Soil Amended with Different Fungi

Experimental Design. Fungal species, inoculum density (ID) and soil type were main factors in a factorial experiment. Three fungal types at three ID in two soil types were replicated 4 times, giving a total of 72 experimental units, which were placed in an incubator in a randomized complete block design.

Materials and Procedures. Fusarium moniliforme, Rhizoctonia solani and Pythium arrhenomanes were cultured in one 1. Erlenmyer flasks on an autoclaved mixture of 150 g of sand, 20 g of cornmeal and 50 ml of distilled water. The cultures were incubated at 25 C for 10 days prior to use.

Different ID were adjusted by mixing the sand and cornmeal cultures with pasteurized or field Houston Black Clay soil at ratios

of 1:10, 1:100 and 1:1000 (V/V). Eight pre-germinated seeds (apparently free from fungal growth) of sorghum hybrid Funk G611 were planted in 190 ml styrofoam cups at a depth of 4.5 cm. Seeds were pre-germinated for 24 hr at 27 C. All cups were completely randomized in Precision incubator set on a 10 - 20 C diurnal cycle (12:12 h).

The cups were flooded for 25 h immediately after planting, and saturated every 48 h while in the incubator. After 9 days, the cups were removed from the incubator and randomly assigned locations on a bench in a greenhouse. The seedlings were watered as needed to maintain uniform moisture in the soil. After 13 days, leaf length, leaf weight, and percent emergence were measured.

Fungicide Seed Treatment in the Field

Experimental Design. Seven fungicide treatments and one control were replicated 5 times in a randomized complete block design, giving a total of 40 experimental units.

Materials and Procedures. Treatments consisted of the following:

Apron 2E (Captan 80WP) - 0.62 kg ai/kg seed (1.5 kg ai/kg seed).
Captan 80 WP - 1.5 kg /kg seed.
Vitavax 200 75 WP - 2.5 kg ai/kg seed.
Apron 2E - 0.5 kg ai/kg seed.
Aliette 80 WP - 10.0 kg ai/kg seed.
Baytan 150F - 1.0 kg ai/kg seed.
Captan 50 WP - 2.0 kg ai/kg seed.

The first two treatments were commercially applied to the sorghum hybrid Funk G611. The experimental hybrid (ATx623 x RTx430) was used for the other treatments.

For the laboratory fungicide applications, 20 g of seed were placed in a 500 ml beaker along with the appropriate mount of fungicide, which had either been suspended or diluted in 3 ml of water. The seed/fungicide mixture was periodically shaken as it dried under a vacuum hood. This technique provided uniform coverage with minimal loss of the fungicide.

Two hundered seeds were planted by hand in 6 meter rows (on one m centers) in Hillsboro, Texas on April 9, 1983. Total number of emerged seedlings was recorded one month later.

Fungicide Seed Treatment in the Laboratory

Experimental Design. Three treatments were replicated 4 times in a completely randomized design, giving a total of 12 experimental units.

Materials and Procedures. Untreated and Apron-treated seed of sorghum hybrid Funk G611 were planted 4.5 cm deep in both field and steamed Houston Black Clay soil in 190 ml styrofoam cups and randomly placed in an incubator (10 - 20 C diurnal cycle 12:12 h). The Apron treatment was commercially applied over the standard Captan and heptachlor treatments. Soil was brought to saturation for 2 days, then flooded for 24 h in the manner described earlier. After 9 days, the cups were removed to a greenhouse bench, where they were maintained at uniform moisture levels for 13 days. Leaf length, leaf weight and final emergence were then measured.

Resistance Studies

Field Observations

Number of emerged plants and percent damping off were evaluated at a sorghum disease nursery near La Ward, Texas, about 30 days after emergence. "Damping off" was qualitatively assigned to emerged seedlings which appeared to either be dead or have little chance of survival. Severe foliar necrosis and dessication were used to identify plants considered to have little chance for survival.

Several discrete disease "nurseries" (collections of established genotypes), and individual lines were evaluated. For the purpose of this thesis, only a few selected lines are considered.

Samples which were collected in the spring of 1983 (described earlier) were also taken from this field and from fields nearby with similar levels of seedling damping off.

Incubator Test

Experimental Design. Variety and soil type were main factors in a randomized complete block factorial experiment. Twelve different varieties were replicated four times in both steamed and field soil giving a total of 96 experimental units.

Materials and Procedures. The following genotypes were used:

(1) SC283-14	(7) BTX623
(2) SC748-5	(8) SC326-6
(3) Tx430	(9) Kaoliang
(4) Tx7078	(10) BTx378

(5) SC630-11E (11) 77CS2

(6) QL3 (12) BTx399

Seeds of SC283-14, SC748-5, Tx430, SC630-11E, BTx378 and SC326-26 were produced in Lubbock, Texas, in 1982. The remaining genotypes were produced in the Brazos Bottoms in 1982. To minimize the variability attributable to seed quality, seeds were pregerminated for 24 h at 27 C before planting.

Ten pregerminated seeds (apparently free of fungal contamination) were planted in styrofoam cups filled with Houston Black Clay soil in the manner described earlier. The cups were randomized in vertical blocks in an incubator set on a 10 - 20 C diurnal cycle (12:12 h). After 7 days in the incubator, the cups were relocated to a greenhouse bench and maintained at uniform moisture for 13 days before measuring final emergence, leaf length and leaf weight.

In Vitro Inoculation

Experimental Design. Variety and inoculation type were two main factors in two way factorial experiment. Experimental units were replicated 4 times in a completely randomized design.

Materials and Procedures. The 12 varieties listed in the previous experiment (same seedlots) were inoculated in the paper blotter apparatus in the same manner described earlier. Two seedlings were placed in each blotter, and inoculated with a 0.5 cm plug of either PDA-cultured P arrhenomenes or sterile PDA (potato dextrose agar).

Leaf length and total dry plant weight were measured eight days after inoculation. In this case, total dry plant weight represents the weight of the whole seedling (including the mesocotyl) without the remnants of the caryopsis.

Root-rot Ratings

Experimental Design. Twelve sorghum genotypes were evaluated using a completly randomized design. Experimental units, consisting of 200 cc contaners, were replicated 4 times.

Materials and Procedures. Twelve sorghum genotypes were evaluated for resistance to root rot in NFS in the greenhouse. Seeds were planted 4.5 cm deep in Houston Black Clay soil in 190 ml styrofoam cups. Cups were moved daily from a greenhouse to an incubator set at 10 C. The mean duration for each temperature was approximately 12 hr. AT the 4 leaf stage, seedlings were removed from the cups, washed and evaluated for root reddening and/or necrosis. Root rot was assessed according to a 1 - 10 scale (1 = 10% root tissue red or necrotic; 10 = 100% root tissue red or necrotic).. Seedlings were watered daily to maintain high moisture levels (near saturation) in the soil.

CHAPTER III

RESULTS

Epidemiology

Complete Moisture and Temperature Effect

When seedlings were removed from the temperature tank, there was noticeable variation in leaf length. Seedlings grown at 15 C had just begun to emerge, while those grown at 25 C were approximately 15 cm long. Within temperature level groups, plants in the wet and saturated treatments grew most rapidly.

Growth patterns of seedlings changed after they were moved to the greenhouse and exposed to uniform moisture and temperature levels. Seedlings exposed to high moisture (saturation and wet treatments) at any temperature in the temperature tank grew more slowly than plants in the moist treatment and were chlorotic. Eventually, lower leaves of the seedlings from the saturated treatment became necrotic. Seedlings exposed to the dry treatment at any temperature in the temperature tank grew very rapidly on the greenhouse bench, and had green leaves. The highly significant effect of moisture level on leaf length was evident ($P = .0001$, Table 2) at the end of the 12 day period of uniform conditions. There were no significant differences in final emergence of plants among any treatments as indicated by the insignificant F test for the overall model ($P = .1953$, Table 2).

Leaf length mean values for the main moisture effect were examined. Leaf length mean values generally decreased as moisture

increased, with a difference of approximately 4 cm between each of the moisture levels (Table 3).

These data indicated that significant moisture effects occurred at several temperatures and that these effects were more evident with time, despite subsequent favorable moisture and temperature conditions.

Table 2. Relative importance of moisture, temperature and their interaction in the reduction of leaf length and final emergence of sorghum seedlings.

Dependent Variable	Source	F value	P value	R^2
Final emergence	Moisture[1]	2.74	.0783	
	Temperature[2]	2.88	.0495	
	Interaction	0.30	.9319	
	Model	1.45	.1953	.3065
Leaf length	Moisture	46.97	.0001	
	Temperature	2.26	.0924	
	Interaction	0.22	.9580	
	Model	9.28	.0001	.7392

[1] See Materials and Methods for a description of moisture levels.
[2] Temperature levels were 15, 20, 25 c and a 15 - 25 diurnal cycle.

Table 3. Effect of moisture level on leaf length of sorghum seedlings.

Moisture level	Leaf length (cm)
Dry	30.438[1]
Wet	26.750
Saturated	22.000

S.E.D. = 2.955 (df = 36)

Comparing the Biotic and Abiotic Components

The effects of soil type and pre-emergence moisture level in this experiment were most noticeable after seedlings were moved to the uniform conditions of the greenhouse for 15 days. Seedlings grown in NFS were chlorotic and smaller than seedlings grown in PFS. Soil type and pre-emergence moisture level significantly affected both leaf length and leaf weight after 12 days in the greenhouse (Table 4). Since these two factors used the same error term in this statistical model, a comparison of the F values indicated their relative importance in this experiment. For both leaf length and leaf weight, the F value for soil type was approximately 10 times greater than the F value for pre-emergence moisture level (Table 4). No significant differences in final emergence were detected with this model (P = .1144, Table 4).

The visual effects described above are supported by trends in the leaf length and leaf weight mean values for the pre-emergence moisture

level X soil type combinations. Leaf length mean values were generally lower in the NFS than in the PFS (Table 5). In both soils, there appeared to be a reduction in leaf length as flooding was added to the moisture regime, though the magnetude of this reduction was greater in the NFS. At the 3 lower moisture levels in NFS, leaf length was generally inversely related to the quantity of moisture available, but in PFS these moisture levels had no apparent effect on leaf length.

The trend in leaf weight mean values (Table 5) was similar to that described above for leaf length (Table 6). In both NFS and PFS, flooding caused a definite and descernible reduction in leaf weight, respectively. The lower mean values of leaf weight and leaf length in NFS compared to PFS represent the highly significant main effect of soil type determined by the analysis of variance procedure (P = .0001 Table 4).

Measuring the Biotic Component

These data represent the differences between values for seedlings grown in NFS and seedlings grown in PFS in a paired difference experiment. After several days of uniform conditions, seedlings in NFS exposed to pre-emergence flooding or saturation were small with necrotic and chlorotic symptoms but those exposed to drier pre-emergence conditions were not chlorotic or stunted.

Table 4. Relative importance of moisture and soil type and their interaction on the reduction in leaf length leaf weight and final emergence of sorghum seedlings.

Dependent Variable	Source	F value	P value	R^2
Leaf length	Moisture[1]	3.06	.0476	
	Soil[2]	33.02	.0001	
	Interaction	3.52	.0304	
	Model	7.45	.0001	.6873
Leaf weight	Moisture	3.96	.0199	
	Soil	27.62	.0001	
	Interaction	1.09	.3767	
	Model	6.11	.0004	.6405
Final emergence	Moisture	1.57	.2222	
	Soil	3.86	.0612	
	Interaction	1.57	.2222	
	Model	1.90	.1144	.3563

[1] See Materials and Methods for a description of moisture levels.
[2] Pasteurized and field soil.

The complete model used to explain variation in leaf length by variation in pre-emergence temperature and moisture levels was significant at the 1% level (Table 7 pg. 34). The main effects of moisture and temperature were highly significant (P = .0001 for both variables). The interaction was significant at the 5% level (P = .0203).

The complete model for leaf weight was highly significant (P = .001, Table 7). The main effects, pre-emergence moisture level and

Table 5. Effect of different pre-emergence moisture levels and soil types on leaf length of sorghum seedlings.

Moisture level[1]	Leaf length (cm)	
	Steamed soil	Field soil
Moist	19.30	18.95
Wet	20.30	17.70
Saturated	19.10	17.68
Flooded	17.60	14.70

S.E.D. = 1.548 (df = 31)

[1] See Materials and Methods for a description of moisture levels.

Table 6. Effect of different pre-emergence moisture levels and soil type on leaf weight of sorghum seedlings.

Moisture level[1]	Leaf weight ($g \times 10^{-3}$)	
	Steamed soil	Field soil
Moist	54.40	49.09
Wet	61.11	47.25
Saturated	58.70	48.63
Flooded	44.20	32.57

S.E.D. = 5.763 (df = 31)

[1] See Materials and Methods for a description of moisture levels.

[2] Means followed by the same letter are not significantly different according to Duncan's New Multiple Range test.

pre-emergence temperature level, were also highly significant (P = .0001 and .0008, respectively). The interaction of the main effects was slight when leaf weight was measured (P = .1225).

The complete model for final emergence was highly significant (P = .0006, Table 7). The pre-emergence moisture main effect was highly significant (P = .0001), but the pre-emergence temperature effect was not significant at the 5% level (P = .1881).

One error term was used in each of the three models (for leaf length, leaf weight and final emergence), allowing the comparison of F values as a measure of relative importance (within the limitations of the experiment). For all three dependent variables, the F value for the main pre-emergence moisture effect was approximately 4 to 7 times greater than the F value for the main pre-emergence temperature effect.

For each dependent variable, a slight interaction was present (leaf length: P = .0203; leaf weight: P = .1225; and final emergence: P = .0744, Table 7).

The main effects and interactions were also evident upon inspection of the mean values for the various treatment combinations. The major trend apparent in the treatment combination mean values is the reduction in leaf length at the higher moisture levels (Table 8), represented by the high F value for the main moisture effect (Table 7). At the two highest moisture levels there was a greater reduction in leaf length at 15 C than at the other temperatures. In general, however, low temperatures reduced leaf length at the two highest moisture levels. At the low moisture levels, there was little

Table 7. Relative importance of moisture, temperature and their interaction in the reduction in leaf length, leaf weight and final emergence of sorghum seedlings.

Dependent Variable	Source	F value	P value	R^2
Leaf length	Moisture[1]	55.10	.0001	
	Temperature[2]	7.62	.0001	
	Interaction	2.24	.0203	
	Model	11.3	.0001	.7877
Leaf weight	Moisture	21.46	.0001	
	Temperature	5.44	.0008	
	Interaction	1.58	.1225	
	Model	5.53	.0001	.6366
Final emergence	Moisture	9.92	.0001	
	Temperature	1.59	.1881	
	Interaction	1.77	.0744	
	Model	3.02	.0006	.4887

[1] See Materials and Methods for a description of moisture levels.
[2] Temperature levels were 10, 15, 20, 25 C and a 10 - 20 C diurnal cycle.

temperature effect. Negative values in the wet treatment refer to occasions when the value for the variable measured was greater for the NFS-seedlings than for the PFS-seedlings.

The mean differences for leaf weight at all temperature and moisture levels (Table 8) were similar to those described above for leaf length (Table 9). Moisture was the major factor influencing seedling leaf weight. The greatest reduction occurred at 15 C in the

two highest moisture levels, but there was very little temperature effect at the low moisture levels.

The mean differences for final emergence were generally greatest in the two highest moisture levels (Table 10). A high degree of variability, not associated with either moisture or temperature, was also evident in final emergence numbers.

Table 8. Effect of different pre-emergence temperature and moisture levels on leaf length (cm) of sorghum seedlings.

	Moisture			
Temperature (C)	Moist[2]	Wet	Saturated	Flooded
10	1.93	-1.58	3.45	9.40
15	0.03	0.95	9.95	12.08
10-20	0.70	-0.77	6.20	7.80
20	2.03	1.95	5.98	7.75
25	0.03	-1.50	2.58	4.00

S.E.D. = 1.69 (d.f. = 60)
[1] Values represent means of differences between paired treatments (steamed soil - field soil).
[2] See Materials and Methods for a description of the moisture levels.

Table 10. Effect of different pre-emergence temperature and moisture levels on final emergence of sorghum seedlings.

	Final emergence[1]			
	Moisture			
Temperature (C)	Moist[2]	Wet	Saturated	Flooded
10	1.50	3.25	2.50	1.75
15	1.50	0.75	1.25	3.25
10-20	-1.00	1.75	1.25	4.25
20	-1.75	2.50	1.75	2.75
25	-1.00	-0.50	-0.25	4.00

S.E.D. = 1.900 (d.f. = 60)

[1] Values represent means of differences between paired treatments (steamed soil - field soil).

[2] See Materials and Methods for a description of the moisture levels.

Etiology

Isolations from Seedlings Grown in the Greenhouse

Seedlings grown in the temperature tanks were characterized by reddening on the primary root systems and water soaked lesions on the crown roots. Species of Pythium and Fusarium were the most commonly isolated (Table 11). The predominant Pythium species was identified as P. arrhenomanes, according to the key of Waterhouse (46) and the description by Drechsler (10). A large number of Pythium isolates formed no oospores in culture, but otherwise resembled P. arrhenomenes. F. moniliforme was isolated frequently.

In a separate experiment, a greater number of isolates of both Pythium and Fusarium spp. were recovered from adventitous roots than from primary roots (Table 12). The proportion of Pythium spp. isolates was also greater in the adventitous roots than in the primary roots.

Isolations from Seedlings Grown in the Field

Isolations from diseased tissue collected on April 4, 1983 at Victoria, Tx. yielded many different fungi. Species of Pythium, Fusarium, Trichoderma and Curvularia were especially common. In one set of collections from the Victoria area, approximately 70% of the isolates were species of Pythium, the majority of which formed lobulate sporangia. From collections made approximately 10 days later at the same location, isolations yielded a smaller proportion of Pythium spp. and a greater proportion of Trichoderma, Fusarium and

Curvularia spp.

Histology

Squash mounts were made of lesion tissue from both adventitious roots and primary roots of seedling grown in the green house. Signs of phycomyceteous fungi, including aseptate hyphae, oospores and sporangi were consistantly found in association with the lesions.

Sections were made from mesocotyls and primary roots of diseased seedlings collected at La Ward and Victoria, Texas, in the Spring of 1983. Signs of pytheaceous fungi were consistantly seen in the form of frequent aseptate mycelium, and occasional oospores. Septate hyphae, various conidial forms and chlamydospore-like objects were also in or on the tissue of field or greenhouse-grown seedlings.

In vitro Pathogenicity Test

After three days, Pythium spp. caused dark, water-soaked lesions, and reduced leaf length by a significant amount (Table 13 pg. 43). There was no noticeable difference, either in lesion type or leaf length reduction among the three Pythium isolates. Fusarium spp. and F. moniliforme caused no appreciable symptoms or differences in leaf length over that of the control.

Soil Amended with Different Fungi

Every possible interaction among the three factors of soil, ID (inoculum density), and fungal species ("fungi") was significant at the 5% level in explaining variation in leaf length (Table 14 pg. 44).

Table 11. Fungi isolated from seedlings grown at 15 20 and 25 C (21 plates per medium).

Fungi	Number of isolates recovered				
	Media				
	RBS[1]	Water[2]	CPV[3]	NS[4]	Total
Pythium arrhenomenes	1	9	3	-	13
Pythium lobulate spp.	1	1	9	-	11
Pythium spp.	-	-	-	1	1
Fusarium moniliforme	3	2	4	8	17
Fusarium spp.	1	1	2	2	6
Rhizoctonia sp.	-	-	-	-	-
Other	2	3	3	1	9

[1] Rose bengal streptomycin agar.
[2] Water agar.
[3] Cornmeal agar pimarcin vancomycin.
[4] Nash Snyder agar.

Table 12. Fungi isolated from roots of seedlings grown under a diurnal temperature cycle[1] with daily watering.

Fungi	Number of isolates recovered	
	Primary roots[2]	Crown Roots[2]
Pythium arrhenomanes	3	10
Pythium lobulate sp.	2	14
Fusarium moniliforme	6	17
Fusarium sp.	8	8
Other	3	-

[1] See Materials and Methods for a description of the temperature levels.
[2] Four media used - 6 plates per medium.
[3] Water agar only - 21 plates.

Virtually no pattern was visible in the NFS treatments for the effects of either different fungal species or different ID on leaf length mean values (Table 15 pg. 45). In the PFS, a trend in the leaf length mean values with respect to ID was discernable for both P. arrhenomanes and R. solani but not for F. moniliforme. Only the hightest ID (1:10) of R. solani caused leaf length to be as low as the NFS control. A definite inverse reslationship between leaf length reduction and ID was apparent with P. arrhenomanes.

The complete model for soil, fungal species and ID was highly significant in explaining variability in leaf weight (Table 14 pg.

44). The soil x fungi and ID x fungi interactions were significant at the 5% level, but not the soil x ID and soil x fungi x ID interactions. Variability appeared to be greater for leaf weight mean values than for leaf length mean values, making patterns more difficult to discern (Table 15 and Table 16). An inverse relationship between leaf weight and ID, however, was evident for P. arrhenomenes (Table 16).

Variability in final emergence was significantly explained by the complete model (Table 14). As with leaf weight, the soil x fungi and fungi x ID interactions were significant (P = .0011 and .0001, respectively), but soil x ID and soil x fungi x ID were not. The only apparent treatment effect was that of P. arrhenomenes at the highest (1:10) ID (Table 17).

Fungicide Seed Treatment in the Field

Emergence was significantly higher for the Funks G611 hybrid planted in Hill Co., Tx. when treated with Apron (metylaxyl - Ciba Ceigy) and Captan, than when treated with Captan alone (Table 18). In the same experiment, however, treatment with Apron alone did not result in significant differences in percent emergence over other seed treatments using the experimental hybrid. The best treatment on the experimental hybrid was Vitavax (Uniroyal), which was not significantly different from other treatments but was significantly different from the control.

Fungicide Seed Treatment in the Laboratory

Leaf length, leaf weight and final emergence were not significantly affected by a commercial treatment of Apron added to the Captan treatment when seedlings were grown in styrofoam cups in an incubator (Table 19). Steaming the soil did significantly increase leaf length and leaf weight.

Table 13. Effect of different fungal species on leaf length of sorghum line BTx623 after 3 days after inoculation in the blotter apparatus.

Designation	Leaf length
P. arrhenomanes[1]	3.25 a[2]
P. arrhenomanes[3]	3.38 a
Pythium sp. (lobulate)	3.62 a
Fusarium sp.	4.75 b
F. moniliforme	4.94 b
Control	4.86 b

[1] Isolate recovered from wheat roots.
[2] Means followed by the same letter are not significantly different based on Fisher's Protected LSD.
[3] Isolate recovered from sorghum seedling roots.
according to Duncans Multiple Range test.

Table 14. Relative importance of soil type, fungal species, inoculum density (ID) and their interactions in the reduction of leaf length, leaf weight and final emergence of sorghum seedlings.

Dependent variable	Source	F value	P value	R^2
Leaf length	soil[1]	22.21	.0001	
	ID[2]	3.58	.3046	
	Fungi[3]	19.00	.0001	
	Soil X ID	6.06	.0042	
	Soil X Fungi	36.23	.0001	
	Fungi X ID	4.75	.0023	
	S X F X ID	3.38	.0155	
	Model	10.85	.0001	.7735
Leaf weight	Soil	6.68	.0125	
	ID	4.49	.0030	
	Fungi	13.74	.0001	
	Soil X ID	1.54	.2237	
	Soil X Fungi	24.00	.0001	
	Fungi X ID	6.26	.0003	
	S X F X ID	1.08	.3753	
	Model	7.50	.0001	.7026
Final emergence	Soil	2.65	.1091	
	ID	4.59	.0145	
	Fungi	4.96	.0105	
	Soil X ID	2.70	.0762	
	Soil X Fungi	7.79	.0011	
	Fungi X ID	9.16	.0001	
	S X F X ID	1.90	.1237	
	Model	5.12	.0001	.6169

[1] Fusarium moniliforme, Pythium arrhenomanes, Rhizoctonia solani.
[2] IDs used were 1:10, 1:100 and 1:1000 - see Materials and Methods.
[3] Pasteurized and field soil.

Table 15. Effects of soil type, inoculum density and fungal species on leaf length (cm) of sorghum seedlings.

	Inoculum density and soil type					
	1:10		1:100		1:1000	
Fungus	Steamed	Field	Steamed	Field	Steamed	Field
Fusarium moniliforme	21.4	14.2	20.7	13.5	19.3	13.9
Rhizoctonia solani	13.8	14.3	20.9	13.3	19.0	12.9
Pythium arrhenomanes	8.8	15.0	10.9	14.0	15.1	16.3
Control	19.4	13.9[1]				

S.E.D. = 2.302 (d.f. = 54)

[1] Sterile sand and cornmeal was added to field and steamed soil at the 1:10 rate for controls.

Table 16. Effects of soil type, inoculum density and fungal species on leaf weight (g x 10^{-3}) of sorghum seedlings.

	Inoculum density and soil type					
	1:10		1:100		1:1000	
Fungus	Steamed	Field	Steamed	Field	Steamed	Field
Fusarium moniliforme	40.30	23.90	36.70	19.20	38.00	25.10
Rhizoctonia solani	20.50	21.70	43.30	26.40	31.70	20.80
Pythium arrhenomanes	5.50	19.00	14.00	25.10	23.40	33.50
Control	35.00	24.00[1]				

S.E.D = 2.01 (d.f. = 54)

[1] Sterile cornmeal and sand was added the 1:10 rate with steamed and field soil for controls.

Table 17. Effects of soil type, inoculum density and fungal species on final emergence of sorghum seedlings.

	Inoculum density and soil type					
	1:10		1:100		1:1000	
Fungus	Steamed	Field	Steamed	Field	Steamed	Field
Fusarium moniliforme	7.75	7.50	7.25	6.50	7.50	7.25
Rhizoctonia solani	7.50	7.50	8.00	7.75	7.25	8.00
Pythium arrhenomanes	3.25	6.75	7.25	8.00	7.25	7.00
Control	7.30	7.75[1]				

S.E.D. = 0.442 (d.f. = 54)

[1] Sterile sand and cornmeal was mixed with steamed and field soil at 1:10 rate for controls.

Table 18. Effects of different fungicidal seed treatments on percent emergence of sorghum seedlings planted in the spring of 1983 in Hill Co., Texas.

Treatment	Rate[1]	Seed type	Percent emergence
Apron (Captan)[2]	0.62 (1.5)	Funks G611	44.0 a[3]
Vitavax 200 75wp	2.5	Exp. hybrid[4]	36.7 ab
Captan 80wp	1.5	Funks G611	32.3 bc
Apron 2E	0.5	Exp. hybrid	29.3 bc
Aliette 80wp	10.0	"	31.3 bc
Baytan 150 F	1.0	"	31.8 bc
Captan 50wp	2.0	"	32.2 bc
Control[5]	-	"	23.3 c

[1] Rate in g ai/kg seed.
[2] Commercial treatment of Apron (seed treatment formulation of metylaxyl - Ceba Geigy) and Captan.
[3] Means followed by different letters differ significantly according to Duncan's New Multiple Range Test.
[4] Laboratory application of fungicide (see Materials and Methods for description) on sorghum hybrid BTx 623 X RTx430.
[5] No fungicide applied to seed.

Table 19. Effect of seed treatment with Apron[1] on leaf length, leaf weight and final emergence of sorghum seedlings grown in an incubator.

Treatment	Leaf length (cm)	Leaf weight (g x 10^{-3})	Final emergence
Field soil Apron	14.00 a	35.00 a	6.75
Field soil No Apron	13.95 a	29.00 a	8.00
Steamed soil No Apron	22.00 b	73.00 b	7.50

[1] Seed treatment formulation of metylaxyl (Ciba Geigy).
[2] Means followed by different letters differ signifcantly according to Duncan's New Multiple Range Test.

Resistance Studies

Field Observations

The number of plants emerged one month after planting varied greatly among the different genotypes (Table 20). The quantity of seeds planted in each row was determined by weight. Therefore, the original plant populations were not known. There appeared to be little correlation between the number of plants emerged and the percent of post emergence damping off.

BTx378 and QL3 were the most resistant lines with 19 and 26% post emergence damping off, respectively. SC748-5 was considered the most susceptible with 45% post emergence damping off.

Incubator Test

Main effects of variety and soil type were significant at the 5% level for all variables measured on seedlings grown in styrofoam cups filled with Houston Black Clay soil and placed in an incubator (Table 21). Interactions were not significant at the 5% level except for the variety x block interaction for final emergence and total leaf length.

In general, emergence and leaf growth (weight and length) were reduced in the NFS treatments for all variables measured (Table 22). Severel lines consistantly had small differences between NFS and PFS for all three variables, and were considered the most resistant. These lines were: 77CS2, QL3 and BTx399. Tx430, SC283-14 and SC748-5 were the most susceptible materials.

In Vitro Inoculation

Seedlings of 12 sorghum lines were grown in the blotter apparatus and inoculated with an isolate of P. arrhenomanes. Varieties, as one factor and treatments, (inoculated vs noninoculated) as another factor were compared in a factorial experiment. No interaction occurred at the 5% level, though both main effects were significant (P = .0001 for varieties and for treatments). There were varying reactions among varieties with respect to leaf length and plant weight. Based on differences between inoculated and non inoculated treatment mean values, QL3, SC630-11E, Tx430 and BTX623 were the most resistant lines (Table 23). SC748-5, BTx399 and Tx7078 were the most susceptible lines. These differences, however, have not been analysed statistically.

Root-rot Ratings

Root-rot ratings differed significantly for two of the varieties tested (Table 24). Tx7078 and BTx399 had significantly more root rot than most of the other lines. SC748-5 and TX7000 did not differ significantly from the other lines but had larger mean root rot ratings, and were not significatly different from Tx7078.

Table 20. Number of emerged plants (live and dead), percent post emergence damping off and resistance ranking of sorghum seedlings planted at La Ward, Tx. in 1983.

Designation	Emerged plants[1]	Percent damping off[2]	Resistance ranking[3]
BTx378	178	19	6
SC630-11E	83	21	2
QL3	133	26	5
SC326-6	66	33	4
Tx7078	76	37	4
Tx430	21	38	4
BTx623	143	59	4
SC748-5	94	66	1
77CS2	71	45	3
SC283	-	-	-
BTx399	-	-	-
Kaolaing	-	-	-

[1] Number of live and dead plants counted 1 month after planting.
[2] Percent of emerged plants considered dead one month after planting.
[3] See Materials and Methods for a description of the ranking scheme.

Table 21. F, P and R^2 values for main and interaction effects of varieties, soil types and blocks on leaf length, leaf weight and final emergence of sorghum seedlings.

Dependent variable	Source	F value	P value	R^2
leaf length	Variety[1]	3.89	.0012	
	Soil[2]	24.41	.0001	
	Block[3]	6.18	.0019	
	Var X Soil	1.26	.2919	
	Var X Block	.78	.7566	
	Soil X Block	2.02	.1301	.7994
	Model			
leaf weight	Variety	2.74	.0124	
	Soil	45.25	.0001	
	BLock	9.37	.0001	
	Var X Soil	1.10	.3898	
	Var X Block	1.16	.3382	
	Soil X Block	1.29	.2957	.8269
	Model			
Final emergence	Variety	5.20	.0001	
	Soil	124.09	.0001	
	Block	24.54	.0001	
	Var X Soil	1.47	.1904	
	Var X Block	1.00	.4965	
	Soil X Block	8.17	.0003	.9088
	Model			

[1] See Materials and Methods for a list of the 12 sorghum genotypes used.
[2] Steamed and Field soil.
[3] See Materials and Methods for a description of blocks.

Table 22. Differences between means of steamed and field soil treatments among 12 sorghum varieties for leaf length, leaf weight and final emergence.

Designation	Leaf length(cm)	Leaf weight(g x 10^{-3})	Final emergence
BTx378	3.25	24.29	3.00
SC630-11E	7.45	30.54	4.50
QL3	1.03	11.77	1.25
SC326-6	3.10	8.21	4.25
Tx7078	7.45	28.41	4.00
Tx430	10.77	31.80	4.50
77CS2	.45	8.52	2.25
SC748-5	9.10	30.89	3.00
SC283-14	12.75	41.59	5.00
BTx399	1.34	9.02	1.50
BTx623	3.40	17.07	2.50
Kaoliang	1.30	11.91	3.25

Table 23. Differences in leaf length and whole plant weight between inoculated and control treatments for 12 sorghum varieties grown in the blotter apparatus.

Designation	Leaf length(cm)	Leaf weight(g x 10^{-3})
BTx378	0.88	3.25
SC630-11E	0.00	0.90
QL3	0.87	1.77
SC326-6	1.63	0.65
Tx7078	2.89	2.38
Tx430	0.50	-0.13
77CS2	2.00	2.23
SC748-5	4.13	5.17
SC283-14	1.75	2.18
BTx399	2.90	4.05
BTx623	0.50	-0.25
Kaoliang	0.63	-0.09

Table 24. Root damage ratings for 11 sorghum genotypes based greenhouse studies in Houston Black Clay Soil.

Designation	Resistance rating[1]
Tx430	2.20 a[2]
77CS2	2.40 a
SC283-14	2.59 a
Tx2536	2.59 a
SC630-11E	2.79 a
BTx378	2.79 a
SC265	3.40 a
SC748-5	4.00 ab
Tx7000	4.10 ab
Tx7078	5.80 bc
BTx399	7.80 c

[1] Rating scheme based on percent of root system with lesions or general necrosis.
[2] Means followed by different letters are significantly different according to Fisher's Protected LSD.

CHAPTER IV

DISCUSSION

Epidemiology and Etiology

Correlation Between Greenhouse Studies and Field Observations

The second experiment in the epidemilogical section was designed to compare the effects of moisture on seedling leaf length, leaf weight and final emergence in both PFS and NFS. The significant soil type x moisture level interaction for leaf length indicates the indirect nature of the deleterious moisture effect (Table 4, pg. 31). High moisture levels had little effect on leaf length in the absence of soil-borne pathogens, but were detrimental when these organisms were present in the soil. The deleterious effects of soilborne microorganisms is indirectly supported by numerous reports of improvement in stand establishment as a result of fungicidal seed treatment (1, 3, 4).

The moisture level X soil type interaction for leaf weight was not significant at the 5% level (Table 4, pg. 31). The lack of significance probably was a result of the level of severity of the test. When the differences between PFS and NFS-grown seedlings were evaluated in another experiment, the interaction between soil type and moisture level for leaf weight was indirectly evident. In this case, the significant moisture main effect ($P = .0001$, Table 7, pg. 34) implies that variation in moisture levels affected seedlings differently in each of the two soils. Thus, there was a soil type x

moisture level interaction. Otherwise, all of the experimental units would have been statistically equal, and the overall main moisture effect would not have been significant. These data, in addition to those from the moisture level X soil type factorial experiment discussed above, showed the importance of soil-borne micro-organisms (pathogens) in the reduction of seedling leaf length and leaf weight.

The extrapolation of these epidemiological results to field situations requires the acceptance of one fundamental assumption. Leaf length, leaf weight and final emergence, as measured in these experiments, must be accurate estimators of the type of subterranean damage which results in pre and post emergence damping off in the field. Final emergence, as measured in these experiments, was less useful than the other two variables. Unexplained variablilty, partly associated with seed quality, made final emergence a less desireable variable than leaf weight or length for this type of research.

An association can be drawn between greenhouse results (measured by leaf length and leaf weight) and field seedling disease (measured by pre and post emergence damping off) through similarities in symptoms and causal agents. In both greenhouse and field, symptoms on root systems and mesocotyls are characterized by water soaked, clear areas bordered by dark rings. Although mesocotyl lesions in the field often appear wrinkled due to dessication, upon rehydration, they closely resemble those on seedlings from PFS in the the greenhouse, and those produced by *in vitro* inoculation in the blotter apparatus.

Both field seedling disease and greenhouse-induced reductions in leaf length and leaf weight appear to be caused by species of *Pythium*.

In greenhouse experiments, Pythium spp. are reported to cause post emergence damping off in small pots (34) and in flats (22). The absence of post emergence damping off in our experiments was probably due to the cessation of treatment conditions followed by exposure to uniform moisture and temperature conditions, favorable for seedling growth. This experimental procedure allowed diseased seedlings to recover and avoid post emergence damping off.

Post emergence damping off is a complex phenomenon combining root and mesocotyl parasitism and extreme environmental conditions unfavarable for sorghum growth. One example of unfavorable environmental conditions would be continued low temperatures and high soil moisture, which could promote seedling disease by slowing plant growth and favoring the parasitic activity of a pathogens. If this environment is of sufficient duration early in seeding establishment, the plant would be killed directly by the pathogen.

Seedling death due directly to parasitic activity in very moist cool conditions is probably more common in greenhouse experiments than in the field. Leukel and Martin (23) and Pratt and Janke (35) experimentally induced post emergence damping off when conditions favorable for seedling disease were extended longer than in any of the experiments in this thesis.

In the field, however, the relationship between the parasitic activity of the fungus and post emergence damping off is probably less direct. Assuming that the superficial layers of the soil dry quickly after temperatures rise in the spring, seedlings which have not yet produced adventitious roots depend on the primary root system. If the

primary roots and mesocotylcannot conduct enough water to meet leaf transpirational demand (due to pathogenic activity), plants more easily succumb to dessication. The foliar symptoms of seedling disease in the field are often characterized by extreme leaf tissue dehydration. Although fungal activity may decrease or cease, diseased seedlings cannot recover under field conditions as they did under the favorable conditions of the experiments described in this thesis.

Undoubtedly, postemergence damping off is closely related to environmental conditons, and also dependent upon severity of seedling damage. If parasitism occurs but conditions are favorable for plant growth, damping off will not ensue. Therefore, parasitism in the greenhouse can be measured by the reduction in leaf length or leaf weight, and this should accurately estimate similar parasitism in the field which leads to seedling death.

Moisture and Temperature

In our epidemiological studies, leaf length and leaf weight reductions demonstrate that moisture was an important factor in the promotion of seedling disease. The main moisture effect on leaf length had an F value approximately 20 times greater than the main temperature effect, when seedlings were grown in NFS-filled trays in the temperature tank (Table 2 pg. 28). In another experiment, the moisture effect F value was about 7 times greater than that for the temperature effect, when the differences between NFS-grown seedlings and PFS-grown seedlings were analysed (Table 7 pg. 34).

The relative importance of moisture in these experiments can be extrapolated, with certain qualifications, to field situations. Given that the the levels of moisture and temperature used in these experiments may differ in range, duration and variablility from those generally found in nature, similar F values for the temperature and moisture factors in these experiments would have meant that little could be inferred about the relative role of either factor in the field. The influence of moisture on the variables measured, however, was so significant in these studies that it is illogical to assume that it would not also exercise an important influence in the field.

Historically, soil moisture has been evaluated for its general role in seedling emergence and stand establishment (12, 29), but only as a direct effect. Researchers have generally been interested in the effects of limited moisture on seed germination and seedling emergence. The relationship between high soil moisture, sorghum seedlings and the soil microflora has not been given adequate consideration.

The importance of moisture in sorghum seedling disease is supported by several observations. In greenhouse containers, which must be watered regularly, surface layers are repeatedly brought to or near 0 bars matric potential. New adventitious roots must grow through these soils where activity by Pythium species is favored by the high moisture levels (9). Adventitious roots of greenhouse-grown seedlings which are watered frequently, often have a high incidence of watered soaked lesions, especially near the root tips. Lobulate Pythium spp. and Fusarium spp. were easily isolated from these

lesions.

The relationship between P. arrhenomanes activity and free soil moisture availability was reported by Martin (26) who found that he could readily isolate the organism from sugarcane rootips in the field after a rain. He also noted that waiting only a few days after the rain resulted in a much higher proportion of Trichoderma and Fusarium isolates. Martin speculated that the organisms act as saprophytes on the tissue killed by P. arrhenomanes

The small and sometimes insignificant temperature effects reported in this thesis do not imply that temperature is of no consequence in nature. Rather, the data show that temperature is not the only or, perhaps, not even the primary factor affecting the establishment and survival (as related to seedling disease) of early-sown sorghum seedlings. Sorghum has been described in several reports as a crop which does not produce a good stand in cool soils.

In addition to previous research, one important natural conincidence has reinforced the image of sorghum as a plant which cannot produce a stand in cold soils. Low spring temperatures are usually associated with an unexpected storm system and high soil moisture. Rains which last continuously or intermittantly for days will maintain moisture at matric potentials near 0 for periods longer than those used to flood seedlings in the experiments described in this thesis. Low temperatures associated with overcast conditons will also reduce evaporation.

Etiology

Pythium spp. appear to be the primary soil-borne pathogens of sorghum seedings in both the greenhouse and field. They were often found in association with necrotic subterranean plant tissue. Fusarium spp., Rhizoctonia spp. and other unidentified fungi were also isolated frequently, but were not as active in pathogenicity tests. Two P. arrhenomanes isolates and 1 lobulate Pythium isolate caused water-soaked lesions on the primary roots of seedlings and significantly reduced leaf length (Table 13). F. moniliforme and Fusarium sp. produced slight to no reddening on the roots, and did not appear to inhibit seedling growth. Minute auxiliary roots grew directly through the agar infested with F. moniliforme with no noticeable damage to the root tissue. Pythium spp. were most pathogenic on young succulent tissue, especially near the root tips. Root tips and succulent tissue are generally considered to be the primary infection courts for Pythium spp. (28).

The relative inability of Fusarium spp. to damage sorghum seedling roots was puzzling, considering earlier reports that described it as a soilborne pathogen of sorghum seedlings (44).

Resistance

The F value for the variety by soil interaction in the incubator study is one of the most important statistics associated with this experiment (Table 22). A highly significant soil X variety interaction in this case would have indicated that varieties differed in their reactions to soilborne micro-organisms. For leaf length,

leaf weight and final emergence, the variety X soil interactions were not significant at the 5% level, but had F values greater than 1. Some statisticians argue that insignificant interactions with F values greater than 1 deserve further examination (39).

The insignifcant soil X variety interaction, in this case, implies that the differences among varieties in the PFS should follow the same pattern as the differences among varieties in the NFS. Thus, subtracting the NFS values from the PFS values for any dependent variable should render 12 values (the number of varieties tested) which are statistically equal. These numbers actually vary considerably (Table 22 pg. 54), for all three dependent variables. An excessive amount of unexplained variablity must have entered into the model error term in order for these differences to exist without the soil X variety interaction. This unexplained variability may have resulted from differences in seed quality which were not eliminated by using pregerminated seed. There was considerable variability in final emergence of plants among PFS treatments, when one would have expected a high degree of uniformity using pregerminated seed.

Given the overall insignificance of the interaction described above, the different values for the three dependent variables, as well as the rankings for leaf length, (Table 25 pg. 66) should be studied for their potential biological value. They do not have statistical reinforcement.

The Blotter Technique

In this factorial experiment, the treated varieties (inoculated) were compared with the untreated varieties (not inoculated) by considering varieties as one factor and treatment (inoculation vs non inoculation) as another factor. A significant interaction would have implied differential reactions to inoculation with P. arrhenomanes. The interaction was not significant. The insignificance was probably due to unexplained variablity in the model error term, since differences between inoculated and uninoculated seedlings for leaf length varied greatly (Table 23). Thus, ranking the varieties based on the differences between treatment types may be biologically informative but can not be supported statistically.

Resistance Rankings

A comparison of the rankings for the four resistance tests leads to interesting speculations about the nature of seedling disease resistance. Three lines were relatively resistant in all or 3 of the tests: BTx378, QL3 and SC630-11E (Table 25). All three are kafirs (caffrorum) and are adapted to temperate climates. SC748-5, which is a tropically adapted zera-zera sorghum, was relatively susceptible in all tests.

These kafirs all have some degree of resistance to Peronosclerospora sorghi the causal agent of sorghum downy mildew. P. sorghi is phylogenetically related to Pythium spp. Resistance to both types of fungi may indicate similar infection and defense mechanisms in the host/parasite interaction for both pathogens.

Table 25. Resistance rankings for 12 sorghum genotypes based on field, incubator, blotter apparatus and greenhouse trials.

Designation	Resistance rankings[1]			
	Field	Incubator[2]	Blotter[3]	Root rot[4]
BTx378	6	4	5	4
SC630-11E	6	2	6	4
QL3	5	5	5	-
SC326-6	4	4	3	-
Tx7078	4	2	1	2
Tx430	4	1	5	4
77CS2	3	6	3	4
SC748-5	1	2	1	3
SC283-14	-	1	3	4
BTx399	-	5	1	1
BTx623	1	4	5	-
Kaoliang	-	5	5	-

[1] See Materials and Methods for a description of ranking scheme. means for each variable.
[2] Ranks based on values for leaf length.
[3] Ranks based on values for leaf length.
[4] Ranks based on values for root damage.

If these kafirs possess heritable resistance traits to Pythium spp. and, therefore, are better adapted to emergence in cool wet soils, then they may prove useful in a breeding program. Screening for these resistance traits, however, will require careful attention to abiotic and biotic factors.

Any screening technique for seedling disease resistance must include the use of field soil and pasteurized soil as a check. Moisture must be controlled and maintained at high levels throughout the test, and flooding for a period for 24-48 hours would be advisable. Though temperature may not be as critical as moisture, a

range between 15 - 20 C throughout the emergence period should give a close approximation of field conditions. USA

LITERATURE CITED

1. Agarwal, BV. K., Verma, H. S. and Singh, O. V. 1977. Treatment of sorghum seeds to control seed-borne fungi and improve emergence. Bull. Grain Tech. 15:118-120.

2. Anderson, A. M. 1947. Cause of reddening of roots and effect of fungi on sorghum seedlings. Proc. Assoc. Off. Seed Anal. 42:117-141.

3. Anzalone, L., Jr. 1981. Efficacy of seed treatment on sorghum seedling emergence in Lousiana. Fungicide and Nematicide Tests 36:161.

4. Anzalone, L., Jr. 1982. Effect of seed treatment on sorghum seedling emergence in Louisiana. Fungicide and Nematacide Tests 37:170.

5. Baker, K. L. 1962. Thermo therapy of planting material. Phytopathology 52:1244-1254.

6. Blum, A. 1969. Seedling emergence and establishment of Sudan grass varieties and sorghum by Sudan grass hybrids under suboptimal temperatures. Isr. J. Agric. Res. 19:101-104.

7. Castor, L. L. 1981. Grain mold histopathology, damage assessment, and resistance screening within Sorghum bicolor (L.) Moench Lines. Dissertation. Texas A&M University. 177 pp.

8. Converse, R. H. and Nagel, C. M., July 15, 1953. Sorghum seed treatment in South Dakota. Plant Dis. Rep. 37:401-403.

9. Donaway, J. M. 1979. Water relations of water molds. Ann. Rev. Phytopathology. 17:431-460.

10. Drechsler, C. 1936. Pythium graminicola and Pythium arrhenomanes. Phytopathology 26:676-683.

11. Elliot, C., Melchers, L. E., Lefebure, C. L., and Wagner, F. A. 1937. Pythium root rot of milo. J. Agric. R. 54:797-834.

12. Evans, W.F., Stickler, F.C. and Lande, H.H. 1961. Sorghum seed germination as affected by moisture and temperature. Kans. Acad. Sci. Trans. 64:210-217.

13. Freeman, T. E., Luke, H. H. and Sechler, D. T. 1966. Pathogenicity of Pythium aphanidermatum on grain crops in Florida. Plant Dis. Rep. 50:292-294.

14. Harris, H. B. and Luttrell, E. S. 1955. Grain sorghum seed treatment tests and diseases in Georgia for 1954. Plant Dis. Rep. 39:329-331.

15. Harris, M. R., and Goss, W. L. 1934. Seedling disease of sorghum and Sudangrass. Mon. Bull. Calif. Agr. Dept. 23:109-118.

16. Hart, R. H. and Wells, H. D. 1965. Effect of temperature and soils on emergence of summer annual forage grasses. Agron. J. 57:636-637.

17. Hendrix, F. F., Jr. and Campbell, W. A. 1973. Pythiums as plant pathogens. Ann. Rev. Phytopath. 11:77-79.

18. Horrocks, M. W. 1972. Model for predicting emergence of grain sorghum. Crop Sci. 14:365-367.

19. Johnson, D. L., Davison, A. D. and Stanley Heathman, E. 1966. A Fusarium root rot of Sorghum vulgare. Phytopathology 56:148-149.

20. Josifovich, J. A. 1967. Germinacion de semillas de sorgos forrajeros en suelo y en cajas de Petri a differentes temperaturas. Rev. Invest. Agropec. B. Aires. Ser. 2 4: 453-473.

21. Junejo, U. A .K. and Malik, M. M. S. 1967. Studies on microflora associated with sorghum seed. 1. Surveys, isolations, and pathogenicity. West Pak. J. Agric. Res. 5:81-92.

22. Kanemasu, E. T., Bark, D. L. and Choy, E. C. 1975. Effect of soil temperature on sorghum emergence. Plant and Soil 45:411-417.

23. Leukel, R.W. and Martin, J.H. 1943. Seed rot and seedling disease of sorghum. USDA Tech. Bull. 839. 26 p.

24. Luttrell, E. S., Crowder, L. V. and Wells, H. D. 1955. Seed treatment tests with perl millet, sudan grass and browntop millet. Plant Dis. Rep. 39:756-761.

25. Martin, J. H., Taylor, J. W. and Leukel, R. W. 1935. Effects of low temperature and depth of planting on emergence of sorghum seedlings in the greenhouse. J. Am. Soc. Agron. 27:660-665.

26. Martin, J. R. 1937. Pathology. Pages 28-35 In Proc. Hawaiian Sugar Planters Assc. 1936. (Rev. Appl. Mycol. 15:561).

27. McCarter, S. M. and Littrell, R. H. 1970. Comparative pathogenicity of Pythium aphanidermatum and Pythium myriotylum to twelve plant species and intraspecific variation in virulence. Phytopathology 60:264-268.

28. Miller, C. R., Dowler, W. M., Peterson, D. H. and Ashworth, R. P. 1966. Observations on the mode of infection of Pythium ultimum and Phytophthora catorum on young roots of peach. Phytopathology 56:46-49.

29. Monk, R. L. 1977. Characteristics of grain sorghum emergence. M.S. Thesis. Texas A&M University 82 pp.

30. Mustain, B. C. 1981. Germination and emergence of grain sorghum [Sorghum bicolor (L.) Moench] at low and high temperatures: Maternal and hybrid effects. Ph.D. Thesis Texas A&M University. 142 pp.

31. Narasimhan, K. S., and Rangaswami, G. Influence of mould isolates from sorghum grain and viability of the seed. Curr. Sci. 16:389-399.

32. Phillips, J. C. and Youngman, V. E. 1971. Effect of initial seed moisture content on emergence and yield of grain sorghum. Crop Sci. 11:354-357.

33. Pinthus, M. J. and Rosenblum, J. 1961. Germination and seedling emergence at low temperatures. Crop Sci. 1:293-296.

34. Porter, R. H. 1956. Rhizopus oryzae Went et Geerlings associated with injury to sorghum seed. Plant Dis. Rep. 40:141.

35. Pratt, R. G. and Janke, G. D. 1980. Pathogenicity of three species of Pythium to seedlings and mature plants of grain sorghum. Phytopathology 70:766-771.

36. Richards, L. A. 1965. Physical conditions of water in soil. In Methods in Soil Analysis. Black, C. A. ed. Amer. Soc. Agron. Madison. pp. 128-152.

37. Russel, G. E. 1978. Plant Breeding for Pest and Disease Resistance. Butterworths, London. 485 p.

38. Singleton, L. L. and Ziv, O. 1981. Effects of Pythium arrhenomanes infection and root-tip amputation on wheat seedling development. Phytopathology 71:315-319.

39. Snedecor, G. W. and Cochran, W. G. 1980. Statistical Methods. Iowa St. Univ. Press. Ames. 507 pp.

40. Sprague, R. and Atkinson, R. E. 1942. Cross inoculation with Pythium arrhenomanes from cereals and grasses in the northern great plains. (Abstract). Phytopathology 32:17.

41. Stanghellini, M. E. 1976. Spore germination, growth and survival of Pythium in soil. Proc. Am. Phytopathol. Soc. 1:211-214.

42. Stoffer, R. V. and Van Ripper, G. E. 1963. Effect of soil temperature and moisture on the physiology of sorghum. Agron. J. 55:447-450.

43. Swanson, A. F. and Hunter, R. 1936. Effect of germination and seed size on sorghum stands. J. Am. Soc. Agron. 28:997-1004.

44. Tarr, S. A. J. 1962. Seed rot, seedling disease, and seed treatment. Chapter 2. In Diseases of sorghum, sudangrass and broomcorn. The Commonw. Mycol. Inst., Kew. Serwey, U.K.

45. Tsao, P. H. 1970. Selective media for isolation of pathogenic fungi. Ann. Ref. Phytopathology. 8:151-186.

46. Waterhouse, G. .M. 1968. The Genus Pythium Pringsheim. Commonw. Mycol. Inst. Mycol. Papers 110:1-71.

47. Williams, R. J. and Rao, K. N. 1981. A review of sorghum grain molds. Trop. Pest Manag. 27:200-211.

VITA

I, Gregory Allan Forbes, was born December 2, 1954, to Ronald and Edythe Forbes. After graduating from Perry High School in Massillon, Ohio in 1973. I worked in construction and other jobs for several years in Clarksville, Tennessee. Later, I attended Murray State University in Murray, Kentucky, graduating with a B.A. degree in Agronomy in 1980. The same year, I accepted a graduate research assistantship at Texas A&M University. One year later, I went to the INTA Experiment Station in Manfredi, Cordoba, Argentina and spent 10 months as a visiting researcher. I returned to Texas in 1982 to complete my M.S. in Plant Pathology.

Permanent address:
Gregory Allan Forbes
805 Woodlawn N.W.
Canton, Ohio 44708